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Kinetic model of pH effect on bioremediation of crude petroleum contaminated soil. 1. model development

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Abstract: Successful design and operation of a bioremediation process for soil contaminated with crude petroleum requires an in-depth understanding of the type of microorganisms involved, the specific reaction they perform, the factors that affect their performance, and their bioremediation kinetics. This paper attempts to develop the kinetic model for the pH being one of the major environmental factors that influence the bioavailability of contaminants, the availability of other nutrients, the activity of biological processes and hence the overall bioremediation kinetics of crude-petroleum contaminated soil. The pH model have been developed at the chemical and mathematical level with the basic assumptions that i) all side chains necessary for catalysis are in the correct protonation state; ii) an enzyme can exist in three degrees of protonation; iii) only one form of the enzyme is capable of binding substrate and catalyzing the reaction; and iv) the substrate is in great enough excess such that the equilibrium constant for the protonation of the free enzyme is the same as for the enzyme-substrate complex. The resulting model equations enable to obtain values of the equilibrium constants (K_1 and K_2) which are significant in determination of the optimal pH for bioremediation reaction rate.

Keywords: Bioremediation, Crude-Petroleum, Kinetic Model, pH

1. Introduction

The most widely distributed environmental pollution can be attributed to contamination with petroleum hydrocarbons, caused by tanker accidents, storage tank ruptures, pipeline leaks, and transport accidents. Oil contamination of coastal areas from offshore spills usually occurs in the intertidal zone of beaches as well as the surrounding land and occupies, in most situations, the top 25 cm of soil. Mechanical removal of the oil is essential, but unfortunately, cannot achieve 100% removal, and some of the oil remains entrapped in the beach matrix or the vados zone of the soil respectively.

Bioremediation of soils contaminated with petroleum hydrocarbons has been established as an efficient, economic, versatile, and environmentally sound treatment[1-5]. This alternative reclamation technique exploits the ability of microorganisms to degrade and/or detoxify organic compounds, and attempts to accelerate natural biodegradation rates. The technique is based on optimization of biological processes to remediate or to minimize the concentration of hazardous pollutants at contaminated sites. The underlying basis of bioremediation of organic pollutants is the detoxification or mineralization of the contaminated species to CO_2 and H_2O. Therefore, this makes it an attractive, environmentally friendly and relatively cost effective alternative to conventional physicochemical techniques, which rely mainly on incineration, volatilization or immobilization of the contaminants[6].

The intensity of hydrocarbon metabolization in soil is influenced by a number of factors, including site-specific (soil properties, temperature, oxygen, pollution history, hydrogeology, geochemistry) and contaminant-specific (composition, concentration, age, bioavailability) factors. Limiting factors need to be overcome if microbial breakdown of contaminants is to be used effectively[7–9]. Optimizing the environmental factors responsible for affecting the progress of bioremediation activity has a crucial role in its success. This may lead to reduced maintenance cost; smooth running of the system year round, successful mineralization of the contaminants, and restoration of the site to a functional ecosystem. This requires understanding of the microorganisms and the conditions necessary for them to become established and maintained, and the scientific data must be translated into

cost-effective, full-scale cleanup processes[10].

For biodegradation rate control models, the distribution and composition of the microorganisms must be determined and an accurate kinetic model that may include environmental or inhibitory parameters must be developed. Factors limiting degradation rates in bioremediation applications need to be adequately identified and addressed. More adequate information and enhanced modeling principles are needed for rational scale-up from the laboratory to the field. Without suitable rate expressions, we cannot design reactors or experiments employing isolated enzymes. Therefore, a thorough exploration of the variables which affect enzyme catalysis and a quantitative analysis of their influence are essential.

The hydrogen ion concentration (pH) has been revealed as one of the important environmental factor that influence the bioavailability of contaminants, the availability of other nutrients, the activity of biological processes, and the characteristics of the contaminants with respect to how they interact with the site's geochemical and geological characteristics.

Soil pH is a measure of the acidity or alkalinity of water. The pH of the environment can significantly affect microbial activity and hence bioremediation rate. Most microorganisms thrive within a neutral range. Bioremediation studies in the laboratory and field have demonstrated that pH ranging from 6.5 to 7.5 is sufficient for optimal bacteria growth of hydrocarbon-degrading microorganisms[11, 12]. However many acidic or alkaline soils supports a viable microbial populations capable of degrading the crude – oil contaminants. Higher acid or alkaline conditions generally inhibit microbial activity, and most bacteria favour neutral conditions[12]. Nevertheless, bacteria that are well adapted to acidic or basic condition have been reported[13]. For example, the sulphur – oxidizing bacteria, an obligate aerobic chemo-autotrophic genus that produce sulphurous acid through oxidation of hydrogen sulphide (H_2S), has been found to function well at pH value of 1. According to the pH ranges in which they function best, bacteria have been classified as neutrophiles, acidophiles, or alkaliphiles respectively. Neutrophiles grow in the range of pH 5 – 8 with optimal growth near neutral pH of 7. Acidophiles grow optimally at a pH below 5.5, while alkaliphiles grow optimally at a pH above 8.5.

The soil pH also affects the solubility of phosphorus, an important nutrient for microbes, and the transport of hazardous metals in soil. Phosphorus solubility is maximized at a pH level of 6.5 and metal transport is minimized at a pH level greater than 6[14]. Furthermore, the pH has a profound affect on biotic contaminant reactions within the soil. Depending on the specific characteristics of the soil, changes in pH may cause materials (i.e., metals) within the soil to precipitate and may increase the mobility of hazardous contaminants present in the soil such as crude-petroleum.

Alternatively, a change in pH may cause the contaminant to become strongly adsorbed to the soil, thus inhibiting degradation. Consequently, pH adjustment may be required. However, it has been suggested by various authors[12, 13, 15], that the adjustment should not be employed unless an associated increase in the biodegradation rate is first demonstrated, and only if the pH control is deemed feasible during remediation. A situation where a pH adjustment is required to optimize a particular microbial population additives such as hydrochloric acid, sulphuric acid, liquid ammonium polysulphide, aluminum and iron sulphates have been used when acidification is required. In the case where increase is pH is required, liming has been carried out using such compounds as calcium oxide (lime), calcium hydroxide, calcium carbonate, magnesium carbonate, potassium hydroxide and calcium silicate slags have been applied[12, 16].

2. Modeling the Effect of pH on Enzyme Activity

It has been established earlier that bioremediation involves the use of enzymatic and metabolic activities of microbes to degrade and detoxify contaminants. Although the mechanisms involved may be complicated and varied, some simple equations have been developed to describe the reaction kinetics of common enzymatic reactions. The rate constants for the formation and breakdown of the enzyme-substrate (ES) complex can be described by:

$$[E]+[S]\underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}}[ES]\xrightarrow{k_2}[E]+[P] \tag{1}$$

For most enzymes, the rate of reaction can be described by the Michaelis-Menten equation[17]:

$$\nu_o = \frac{\nu_{max}[S]}{K_m + [S]} \tag{2},$$

where $\nu_o = k_2[ES]$ and $\nu_{max} = k_2 E_T$ with ν_0 and ν_{max} being respectively the initial and maximum reaction rates, and E_T the total concentration of enzymes. The Michaelis constant Km is given by the expression:

$$K_m = \frac{(k_{-1} + k_2)}{k_1} \tag{3}$$

pH has a marked effect on the velocity of enzyme-catalyzed reactions. pH change might affect:

- Enzyme in ways to alter the binding of substrate to enzyme, which would affect K_m.
- Enzyme in ways to alter the actual catalysis of bound substrate, which would affect kcat
- Enzyme by globally, changing the conformation of the protein.
- Substrate by altering the protonation state of the substrate.

Optimal activity of an enzyme is greatly influenced by

the activity of the medium. Enzymes are proteins constructed from various amino acids. Biochemical units possess basic, neutral, or acidic groups. Consequently, the intact enzyme may contain both positively or negatively charged groups at any given pH. Such ionizable groups are often apparently point of the active site since acid- and base- type catalytic action has been linked closely to several enzyme mechanisms. For the appropriate acid or base catalysis to be possible, the ionizable groups in the active site must often each possess a particular charge: That is, the catalytically active enzyme exist in only one particular ionization state. Thus, the catalytically active enzyme may be a large or small fraction of the total enzyme present, depending upon the pH.

3. Model Formulation

How pH affects an enzyme kinetics can be modeled at the chemical and mathematical level. In modeling the effect of pH on enzyme reaction, the following assumptions are made.

- An enzyme can exist in three degrees of protonation (i.e., EH^{2+} = di-protonation or (E^{2-}), EH^{+}= mono-protonation or (E-), and E = free state)
- All side chains necessary for catalysis are in the correct protonation state
- Only one form of the enzyme is capable of binding substrate and catalyzing the reaction, and in this case it is EH+ or E-
- In addition it is assumed in this work that the substrate is in great enough excess such that the equilibrium constant for the protonation of the free enzyme is the same as for the enzyme-substrate complex.

3.1. Reaction Mechanism

Based on the assumptions highlighted above the following mechanism could be proposed for the elucidation of the effect of the hydrogen-ion (h^+) concentration on the enzyme activity upon bioremediation:

$$E \underset{+h^+;\ k_{-1}}{\overset{-h^+;\ k_1}{\Longleftrightarrow}} E^- \underset{+h^+;\ k_{-2}}{\overset{-h^+;\ k_2}{\Longleftrightarrow}} E^{2-} \tag{4}$$

In Equation (4), E^- denotes the active enzyme form, E and E_{2-} denotes the inactive forms, obtained from protonation and deprotonation of the active site of E^-, respectively. k_1 and k_2 are the rate constants for the forward reactions while k_{-1} and k_{-2} represent the respective rate contacts for the backward reactions from the indicated reactions.

We assume further that the first ionization completely eliminates enzyme activity. The ionizations away from the E- state of the enzyme are not considered.

At steady state:

$$\frac{dE^-}{dt} = k_1E + h^+k_{-2}E^{2-} - h^+k_{-1}E^- - k_2E^- = 0 \tag{5}$$

$$\frac{dE^{2-}}{dt} = k_2E^- - h^+k_{-2}E^{2-} = 0 \tag{6}$$

From Equation (6) upon algebraic manipulations it could be deduced that

$$\frac{k_2}{k_{-2}} = K_2 = \frac{h^+E^{2-}}{E^-} \tag{7}$$

K_2 in equation (7) would represent the equilibrium constant for the deprotonation of the active site of E-. From equation (5) we have

$$k_1E + h^+k_{-2}E^{2-} = h^+k_{-1}E^- + k_2E^- \tag{8}$$

But from equation (6), $k_2E^- = h^+k_{-2}E^{2-}$

Plugging in this value into equation (8), it is obvious that the following simplified expression could be obtained upon simple algebraic manipulations:

$$\frac{k_1}{k_{-1}} = K_1 = \frac{h^+E^-}{E} \tag{9}$$

Similarly, K1 in equation (9) would represent the equilibrium constant for the protonation of the active site of E-. We can express the total enzyme concentration ET as:

$$E_T = E + E^- + E^{2-} \tag{10}$$

From Equation (7): $E^{2-} = \frac{K_2E^-}{h^+}$; and similarly from Eqn (9): $E = \frac{h^+E^-}{K_1}$.

The fraction of total enzyme present which is active (f_{E-}) would be the ratio of the active site concentration E-to the total enzyme concentration, i.e.

$$f_{E-} = \frac{E^-}{E + E^- + E^{2-}} = \frac{E^-}{\frac{h^+E^-}{K_1} + E^- + \frac{K_2E^-}{h^+}} \tag{11}$$

or

$$f_{E-} = \frac{h^+K_1E^-}{(h^+)^2E^- + K_1h^+E^- + K_2K_1E^-} = \frac{h^+K_1}{K_2K_1 + K_1h^+ + (h^+)^2} \tag{12}$$

And similarly,

$$f_E = \frac{E}{E + E^- + E^{2-}} = \frac{\frac{h^+E^-}{K_1}}{\frac{h^+E^-}{K_1} + E^- + \frac{K_2E^-}{h^+}} \tag{13}$$

or

$$f_E = \frac{(h^+)^2}{K_1K_2 + h^+K_1 + (h^+)^2} \tag{14}$$

And it could be derived in the same manner that

$$f_{E2-} = \frac{K_1K_2}{K_1K_2 + h^+K_1 + (h^+)^2} \quad (15)$$

To show the influence of pH on the maximum reaction velocity, ν_{max}, in equation (2) above, we replace the total enzyme concentration E_T with the total active form concentration $E_T f_{E-}$.

$$\text{Re}call: \ \nu_{max} = k_2E_T = k_2E_Tf_{E-} \quad (16)$$

Substituting Eqn. (12) into Eqn. (16) we have

$$\nu_{max} = \frac{h^+K_1k_2E_T}{K_2K_1 + K_1h^+ + (h^+)^2} \quad (17)$$

Equation (17) represents the effect of pH on maximum rate of reaction. From Michaeli-Menten equation, (Eqn.2);

$$\nu_i = \frac{\nu_{max}S}{K_m + S} \quad (18)$$

Substituting Eqn. (17) into Eqn.(18), we have the following:

$$\nu_i = \frac{h^+K_1k_2E_TS}{(K_2K_1 + K_1h^+ + (h^+)^2)(K_m + S)} \quad (19)$$

Equation (19) represents the modified Michaeli-Menten equation showing the effect of pH on the rate of biodegradation as affect to νmax.

pH can also affect Km. However, literatures have suggested that if the substrate does not have different ionization states with different affinities for free enzyme and if formation of the enzyme-substrate complex does not influence K1 and K2, then the analysis shows Km is independent of pH. In such cases, pH effects on Km can be assumed relatively insignificant and equation (19) alone can be used to represent the dependence of enzyme-catalyzed reaction rates on pH.

However, the biodegradation of crude oil involve multiple substrate and multiple enzyme processes, and as such there will be different ionization states with different affinities for free enzyme and the formation of the enzyme-substrate complex will influence K_1 and K_2, resulting in K_m been dependent on pH. Therefore, it is imperative to investigate or model the effect of pH on K_m.

3.2. Modeling the Effect of pH on K_m

Recall from equation (3) that $K_m = \frac{k_{-1} + k_2}{k_1}$ On comparing the proposed reaction mechanism (equation (4) with that of the breakdown of the enzyme-substrate (ES) complex (equation (1), it can be further assumed that for negligibly low values of k_{-2} they are identical. Furthermore, the rate constants in equation (4) could be defined as follows:

$$k_1 = \frac{E^-}{E} = \frac{f_{E-}}{f_E} \quad (a);$$

$$k_{-1} = \frac{E}{E^-} = \frac{f_E}{f_{E-}} \quad (b);$$

and

$$k_2 = \frac{E^{2-}}{E^-} = \frac{f_{E2-}}{f_{E-}} \quad (c)$$

(20a, b, c)Substituting Equations (12, 14 and 15) into Eqn. (20a, b, c) we have the following:

$$k_1 = \frac{h^+K_1}{K_2K_1 + K_1h^+ + (h^+)^2} \ \text{x} \ \frac{K_1K_2 + h^+K_1 + (h^+)^2}{(h^+)^2} = \frac{K_1}{h^+} \quad (21)$$

Similarly,

$$k_{-1} = \frac{h^+}{K_1} \ \text{(a) and} \ k_2 = \frac{K_2}{h^+} \ \text{(b)} \quad (22)$$

Substituting Eqns (21, 22a&b) into Eqn (3) we have

$$K_m = \frac{\frac{h^+}{K_1} + \frac{K_2}{h^+}}{\frac{K_1}{h^+}} = \left(\frac{h^+}{K_1}\right)^2 + \frac{K_2}{K_1} \quad (23)$$

Equation (23) represents the effect of pH on K_m for the bioremediation reaction. Clearly evident is the fact that K_m is directly proportional to the square of the hydrogen ion concentration.

Substituting Eqn (23) into Eqn (19) we have

$$\nu_i = \frac{h^+K_1k_2E_TS}{(K_2K_1 + K_1h^+ + (h^+)^2)\left(\left(\frac{h^+}{K_1}\right)^2 + \frac{K_2}{K_1} + S\right)} \quad (24)$$

Moreover, the hydrogen-ion concentration is usually expressed as pH, which is defined as the negative logarithm of the hydrogen-ion concentration. i.e

$$pH = -\log[h^+] \Rightarrow h^+ = 10^{-pH} \quad (25)$$

Thus,

$$\nu_i = \frac{10^{-pH}K_1k_2E_TS}{(K_2K_1 + 10^{-pH}K_1 + (10^{-pH})^2)\left(\left(\frac{10^{-pH}}{K_1}\right)^2 + \frac{K_2}{K_1} + S\right)} \quad (26)$$

Equation (26) represents the modified Michaeli-Menten equation showing the overall effect of pH on the rate of Enzyme-catalyzed reaction. The values of the equilibrium constants (K_1 and K_2) are significant in determination of the optimal pH for the process. We can obtain the values of K_1 and K_2 from graph of experimental data. For the sake of experimental determination, we affirm that the total enzyme concentration E_T is composed of the catalytically active enzyme E- plus the inactive part (either E or E^{2-} as the case may be). Thus, we can assume that

$$E_T = E^- + E \ \text{OR} \ E^{2-} + E^- \text{and} \ E = E^{2-} = E_T - E^- \quad (27)$$

Manipulation of Equation (9) and Equation (27) yields the following:

$$h^+ = \frac{K_1 E}{E^-} = \frac{K_1(E_T - E^-)}{E^-} = \frac{K_1 E_T}{E^-} - K_1 \quad (28)$$

Therefore, a graph of h+ versus ET/E- will give a straight line with a slope of value K1

Similarly, from Equations (7) and (27):

$$h^+ = \frac{K_2 E^-}{E^{2-}} = \frac{K_2 E^-}{(E_T - E^-)} \Rightarrow \frac{1}{h^+} = \frac{1}{K_2}\frac{E_T}{E^-} - \frac{1}{K_2} \quad (29)$$

Again, a graph of 1/h+ versus $E_T/E-$ will give a straight line with a slope of value $1/K_2$.

The values of K_1 and K_2 obtained from the graph of Eqns. (28) and (29) respectively can be used to evaluate the optimum hydrogen-ion concentration (h^+_{opt}) using the relation:

$$h^+_{opt} = \sqrt{K_1 K_2} \quad \text{or} \quad (pH)_{opt} = \tfrac{1}{2}(pK_1 + pK_2) \quad (30)$$

Where pKi is defined as – log Ki[17].

$$h^+_{opt} = 10^{-pH_{OPT}} = \sqrt{K_1 K_2} \quad (31)$$

4. Conclusion

The dependence of enzyme-catalyzed reaction rates on pH has been derived at both mathematical and kinetic levels, with the resulting rate expressions derived as modifications of the Michaelis-Menten equation. Adequate consideration has been given to the fact that the pH could not only influence the maximum reaction rate, but also the Michaelis-Menten constant. The values of the equilibrium constants (K_1 and K_2) which are significant in determination of the optimal pH for the bioremediation reaction could be deduced from appropriate experimental plots based on the model equations derived. Validation of these model equations with experimental data will be the subject of the next paper.

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